

(acetonitrile) according to the following profile: 0-60 min, A 90-60%, B 10-40%, curve = 1; 60-70 min, A 60%, B 40%. Flow rate was 0.6 ml/min at 22°C. The UV detector range was 0.01 AUFS and 202 nm wavelength.

Also, *Panax quinquefolius* berry and root extracts (obtained from Wisconsin) were extracted and analyzed using the same HPLC assay.

*Panax ginseng* root extract was also prepared using the same extraction procedure for the berry extract preparation. The root was obtained from Shanghai Pharmaceutical Company, China. FIG. 2 compares six major ginsenoside concentrations of *Panax ginseng* berry extract and *Panax ginseng* root extract using the same HPLC assay. FIG. 2 demonstrates that the concentrations of ginsenoside Re Rb2 and Rd in *Panax ginseng* berry are significantly higher than the root. Chemical structures of two ginsenosides are also shown in FIG. 1.

## EXAMPLE 2

### PREPARATION OF GINSENOSIDE-ENRICHED AND GINSENOSIDE-FREE *PANAX GINSENG* ROOT FRACTIONS

A procedure was developed at the University of Illinois Functional Food for Health Lab to successfully prepare ginsenoside-enriched and ginsenoside-free fractions from both methanol and water extracts prepared from *Panax ginseng* roots on request from a botanical supplements maker (Leiner Health Products, CA). The extracts were solubilized in water and chromatographed on a column of HP-20 (Diaion) and developed by a reverse gradient solvent system of H<sub>2</sub>O (100%, fraction 1), H<sub>2</sub>O-MeOH (8:2, fraction 2), H<sub>2</sub>O-MeOH (1:1, fraction 3), MeOH (100%, fraction 4), and MeOH-CHCl<sub>3</sub> (1:1, fraction 5). Analysis by HPLC showed fractions to contain 0-60.1% total ginsenosides, and that the profiles of individual ginsenosides vary as follows: fraction 1 was devoid of ginsenosides; fraction 2 gave a total ginsenoside content of 6.0% with ginsenosides Rg1 (54.3%) and Re (35.5%) accounting for 90%; fraction 3 showed a total ginsenoside content of 60.1% with ginsenosides Rg1 (43.7%) and Re (24.4%) accounting for 68.1%;

fraction 4 contained the second highest concentration of ginsenosides at 42.1%, with ginsenoside Rb1 accounting for 47% of the total; and fraction 5 showing trace quantities of ginsenosides. Thus, one of skill in the art will be able to utilize the above method for preparation of ginsenoside-enriched and ginsenoside-free fractions from ginseng berry extract.

### EXAMPLE 3

#### BIOACTIVITY-GUIDED FRACTIONATION AND STRUCTURE IDENTIFICATION/ELUCIDATION

For the isolation of active isolates from active ginseng berry fractions, the materials are subjected to separation procedures employing gravity, vacuum, flash, and low-medium pressure column chromatography employing silica gel as the primary adsorbent will be used in the first order. Other adsorbents or media that may be employed include florisil, alumina, ion-exchange resins, exclusion gel (Sephadex), and C18-bonded silica gel. Preparative layer TLC or semi-preparative and/or preparative high performance liquid chromatography (Prep-HPLC) are used, especially in the isolation and purification of active compounds in the final stages of separation. Other systems of partition chromatography may also be employed as necessary.

The identification of known novel active isolates or the elucidation of their structure is established by measurement and interpretation of their physical (TLC Rf value, HPLC retention time, melting point, and mixture melting point) and spectroscopic (UV, IR, NMR and MS) data. In the identification of known active isolates, comparison of the melting point, optical rotation, UV, IR, <sup>1</sup>H- and <sup>13</sup>C-NMR, low resolution MS, TLC Rf and/or HPLC Rt values to those reported in the literature or to those obtained for reference standards are sufficient. For the elucidation of the structures of novel active compounds, the molecular formula is determined by exact mass measurement using a Q-TDF mass spectrometer) and/or elemental CHO(N) analysis. Tandem mass spectrometric fragmentation patterns coupled with appropriate NMR data allows the determination of functional groups and partial structures. Stereochemistry is determined by use of ORD

and CD coupled with high-field  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectroscopy, with appropriate two-dimensional (2D) and decoupling experiments ( $^1\text{H}$ - $^1\text{H}$  COSY,  $^1\text{H}$ - $^1\text{H}$  DQ-COSY and  $^1\text{H}$ - $^1\text{H}$  HOHAHA (TOCSY) for proton spin systems;  $^1\text{H}$ - $^{13}\text{C}$  HETCOR and HMQC for one-bond C-H couplings; HMBC, selective INEPT,  $^1\text{H}$ - $^{13}\text{C}$  COLOC and  $^1\text{H}$ - $^{13}\text{C}$  FLOCK for long range C-H correlations; NOESY, ROESY and NOE difference experiments for substituent spatial and stereochemical relationships). When necessary, chemical or enzymatic hydrolysis (e.g., ginsenosides and/or polysaccharides), and derivitization (e.g. acetylation, oxidation, reduction) experiments are conducted in conjunction with LC-MS-MS to assist in the determination of the structures.

Conformational analysis may be performed using computer-aided molecular mechanics calculation (e.g. Monte Carlo searching method using MacroModel/Batchmin program (version 4.5) and MM2 forcefield (Sun *et al.*, 1997, Qiu *et al.* 1998). In case of compounds with novel carbon skeletons or posing difficult stereochemistry problems, single crystal X-ray crystallographic analysis is carried out.

#### EXAMPLE 4

##### **PANAX GINSENG BERRY EXTRACT PREPARATION AND ADMINISTRATION**

250 mg of *Panax ginseng* berry extract was dissolved in 50 ml MeOH as solution A. 1,500 mg polyvinylpyrrolidone or PVP-10 (Sigma Chemicals, St. Louis, MO) was dissolved in 50 ml MeOH as solution B. After mixing A and B, the mixture was evaporated under  $\text{N}_2$  to yield 250 mg dried extract at  $50^\circ\text{C}$ . Before each experiment, the dried extract was dissolved in distilled deionized water and vortexed for 2 min at room temperature. The solution was injected intraperitoneally (IP) once a day at a dose of 50 or 150 mg/kg body weight. 150 mg/kg *Panax ginseng* berry extract contains approximately 20 mg/kg ginsenoside Re.

Also, ginsenoside Re was obtained from Shanghai Pharmaceutical Company, China. HPLC analysis was performed to confirm that ginsenoside Re had a purity of >